Targeting mediators of Wnt signalling pathways by GnRH in gonadotropes

REVIEW

Targeting mediators of Wnt signalling pathways by GnRH in gonadotropes

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Abstract

The binding of GnRH to its receptor on pituitary gonadotropes leads to the targeting of a diverse array of signalling mediators. These mediators drive multiple signal transduction pathways, which in turn regulate a variety of cellular processes, including the biosynthesis and secretion of the gonadotropins LH and FSH. Advances in our understanding of the mechanisms and signalling pathways that are recruited to regulate gonadotrope function are continually being made. This review will focus on the recent demonstration that key mediators of the canonical Wnt signalling pathway are targeted by GnRH in gonadotropes, and that these may play essential roles in regulating the expression of many of the key players in gonadotrope biology, including the GnRH receptor and the gonadotropins.

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Introduction

GnRH occupancy of GnRH receptors leads to the activation of multiple signal transduction pathways (Naor 1997, 2009, Naor *et al.* 2000, Millar *et al.* 2004, 2008, Caunt *et al.* 2006, Dobkin-Bekman *et al.* 2006). In gonadotropes, GnRH activates phospholipase-Cβ via coupling to $G_{q/11}$, resulting in the hydrolysis of membrane-bound phosphatidylinositol 4,5-bisphosphate into inositol 1,4,5-triphosphate and diacylglycerol, which respectively mobilise intracellular Ca^{2+} and activate protein kinase C (PKC). The diverse mechanisms and intracellular signalling pathways that have been reported to contribute to the regulation of gonadotrope function in response to GnRH stimulation have been reviewed extensively (Millar *et al.* 2004, 2008, Pawson & McNeilly 2005, Naor 2009), and will not be discussed further here. Instead, this review will focus on the proposed roles of Wnt signalling mediators in regulating gonadotrope function in response to GnRH.

A 'rough guide' to Wnt signalling

Wnt signalling plays an important role in embryonic development influencing cell proliferation, survival and differentiation (Huelsken & Behrens 2002, van Es *et al.* 2003, Moon *et al.* 2004, Nelson & Nusse 2004). Aberrant Wnt signalling can lead to a range of diseases, most notably cancer (Polakis 2000). The canonical Wnt/β-catenin pathway is perhaps the best described Wnt signalling pathway. The key effector of this pathway is β-catenin. In the absence of Wnt ligand (19 family members in humans) stimulation of the Frizzled (FZD) family of receptors (10 family members in humans), cellular β-catenin levels are kept very low. This is because β-catenin is held in a destruction complex, which includes amongst others adenomatous polyposis coli, axin, glycogen synthase kinase 3 (GSK3) and casein kinase 1 (CK1). GSK3 hyperphosphorylates β-catenin, thereby targeting it for ubiquitination and subsequent degradation via the proteasomal degradation pathway. When Wnts bind to and activate the FZD receptors, GSK3 activity is inhibited, allowing the levels of β-catenin to stabilise, and β-catenin translocates to the nucleus where it acts as a co-factor to T cell factor (TCF)/lymphoid enhancer factor transcription factors to promote the transcription of Wnt target genes, many of which are key in developmental processes (Huelsken & Behrens 2002, van Es *et al.* 2003, Moon *et al.* 2004).

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Less well characterised are the so-called non-canonical Wnt pathways, several of which have been proposed over the past 20 years, including the Wnt/Ca^{2+} pathway and Wnt/c-Jun N-terminal kinase (JNK) pathway (Kuhl *et al.* 2000, van Es *et al.* 2003, Veeman *et al.* 2003, Kohn & Moon 2005). These pathways are also activated by Wnt ligands binding to FZD receptors, and are thought to influence processes such as cell polarity, cytoskeletal reorganisation and cell movement, and utilise a diverse array of signalling mediators and transcription factors to drive these events, including PKC, Ca^{2+}/calmodulin (CaM), calcineurin (CaN), Ca^{2+}/CaM-dependent kinase II, nuclear factor of activated T cells (NFAT), dishevelled, RhoA, Rac, Cdc42 and JNK (Kuhl *et al.* 2000, van Es *et al.* 2003, Veeman *et al.* 2003, Kohn & Moon 2005, Katoh & Katoh 2007).

Although there are two isoforms (α and β) of GSK3, it is GSK3β that phosphorylates β-catenin, and targets it for ubiquitination and proteolytic degradation in the inactive canonical Wnt/β-catenin signalling pathway (Frame & Cohen 2001). Following activation of Wnt/β-catenin signalling, GSK3β is inhibited by a poorly defined mechanism, thereby allowing the stabilisation and accumulation of β-catenin levels. GSK3 was originally identified as a key mediator of insulin signalling, and is now thought to be involved in many other signalling pathways with a diverse array of proposed substrates, including Tau, CREB, NFκB, MUC1, cyclin D1, MYC, NFAT and JUN (Frame & Cohen 2001, Grimes & Jope 2001). Multiple kinases have been implicated in the Ser^{9} and Ser^{21} phospho-inhibition of GSK3β and GSK3α respectively, including the phosphatidylinositol 3-kinase (PI3K)–Akt/protein kinase B (PKB) signalling axis (such as that occurring in classical insulin signalling), p90RSK, PKC, protein kinase A (PKA), p70 S6 kinase and extracellular signal-regulated protein kinase (ERK) (Stambolic & Woodgett 1994, Frame & Cohen 2001, Grimes & Jope 2001, Ding *et al.* 2005). It was initially thought that Ser^{9} GSK3β phospho-inhibition was the mechanism of GSK3β inhibition within the Wnt/β-catenin pathway (Frame & Cohen 2001). However, a study using a mouse knock-in of a Ser^{9}Ala GSK3β mutation reported normal embryonic development, suggesting that the Ser^{9} phospho-inhibition of GSK3β is not implicated in canonical Wnt signalling (McManus *et al.* 2005). Both isoforms of GSK3 are probably important for Wnt signalling because deletion of one has no effect, as long as the other is present, possibly suggesting isoform redundancy (Doble & Woodgett 2003). Furthermore, in the mouse knock-in analysis where the $Ser^{9/21}$ residues of GSK3β/α respectively were mutated to alanine residues, normal β-catenin accumulation was observed in response to Wnt3a stimulation, again confirming that Ser^{9} phosphorylation is not the mechanism for inhibiting GSK3β in Wnt/β-catenin signalling (McManus *et al.* 2005). In addition, mutational studies of the 'insulin pool' of GSK3 suggest that insulin-induced $Ser^{9/21}$ phospho-inhibition is not involved in the activation of the Wnt signalling pathway, and that Wnt signalling does not alter the glycogen synthase output of insulin signalling (Ding *et al.* 2000, Ng *et al.* 2009). The 'insulin' and Wnt pools of GSK3β may therefore be considered to be functionally distinct.

A number of theories have arisen regarding the mechanism involved in GSK3β inhibition in the Wnt/β-catenin pathway. Some involve the GSK3β-binding protein called frequently rearranged in advanced T-cell lymphoma (FRAT), which can block GSK3-induced phosphorylation of β-catenin without affecting glycogen synthase activity in the insulin signalling pathway (Thomas *et al.* 1999). Other theories involve the essential Wnt pathway protein dishevelled (Huelsken & Behrens 2002, van Es *et al.* 2003, Moon *et al.* 2004). It has also been demonstrated that Wnt signalling inhibits GSK3 through a PKC-mediated mechanism (Cook *et al.* 1996), suggesting that PKC may have a role in inhibiting GSK3 within the canonical Wnt/β-catenin pathway, in addition to the phospho-inhibition at $Ser^{9/21}$ such as in classical insulin signalling. CK1 can enhance GSK3 activity by acting as a priming kinase. However, CK1 also has an essential role in positively transducing the canonical Wnt signal, suggesting its involvement in GSK3β inhibition (Doble & Woodgett 2003). Alternatively, different CK1 isoforms may positively and negatively regulate GSK3β activity. Interestingly, GSK3β not only has been implicated in canonical Wnt/β-catenin signalling, but may also play a negative regulatory role in the non-canonical Wnt/Ca^{2+} pathway, in which it is thought to function as a nuclear export kinase, thus terminating NFAT transcriptional activity (Crabtree & Olson 2002, van Es *et al.* 2003, Katoh & Katoh 2007).

The FZD family of receptors are seven-transmembrane-spanning receptors that resemble G protein coupled receptor (GPCRs), and are now listed by the International Union of Pharmacology as a novel and separate family of GPCRs, the 'Class FZD' (Schulte & Bryja 2007). One of the first demonstrations that FZDs signal via G-protein coupling came from the early studies by Malbon *et al.* (Liu *et al.* 1999, 2001). It was demonstrated that β_2-adrenergic receptor/FZD1 chimera bearing the cytoplasmic domains of rat FZD1 was able to stimulate β-catenin stabilisation and β-catenin/TCF transcriptional activity in response to isoprenaline stimulation, and that this was inhibited when certain α-protein α-subunits ($G_{\alpha o}$ and $G_{\alpha q}$) were depleted by antisense RNA, or by pertussis toxin pretreatment (Liu *et al.* 1999, 2001). Importantly, these and subsequent studies appeared to suggest that non-FZD GPCRs could target β-catenin activity in response to stimulation by their cognate ligands (Malbon 2005).

Targeting Wnt signalling mediators by non-FZD GPCRs

A number of non-FZD GPCRs have been shown to target β-catenin/TCF activity in response to their cognate ligands, including the prostanoid receptors (Fujino & Regan 2001, Fujino *et al.* 2002), M1 muscarinic acetylcholine receptor (Farias *et al.* 2004), lysophosphatidic acid (LPA) receptor (Yang *et al.* 2005) and thromboxane A2/TP_a receptor (Yan & Tai 2006). The first demonstration of non-FZD targeting of β-catenin/TCF-dependent signalling was through the FP_B prostanoid receptor (Fujino & Regan 2001). Stimulation of FP_B-expressing cells with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) led to reorganisation of β-catenin, a decrease in phosphorylation of cytoplasmic β-catenin and activation of β-catenin/TCF-dependent transcription. In addition to these findings, it was demonstrated that activation of the EP_2 and EP_4 prostanoid receptors by PGE_2 promoted β-catenin/TCF-dependent transcription (Fujino *et al.* 2002). In addition, the EP_2 and EP_4 receptors were demonstrated to target Ser^9 GSK3β phospho-inhibition by a PKA-dependent mechanism (Fujino *et al.* 2002). The M1 muscarinic acetylcholine receptor was demonstrated to inhibit GSK3β activity, stabilise β-catenin levels and induce the expression of the Wnt target genes *en-1* and *cycD1* resulting in the protection of neurons from amyloid-β-peptide neurotoxicity in a rodent model of Alzheimer's disease (Farias *et al.* 2004). The LPA receptor was demonstrated to target β-catenin to induce colon cancer cell proliferation (Yang *et al.* 2005). LPA was shown to promote the nuclear translocation of β-catenin, activation of β-catenin/TCF-dependent transcription, phospho-inhibition of GSK3β and activation of β-catenin/TCF target genes. Furthermore, all these LPA-induced events were apparently dependent on conventional PKC activity (Yang *et al.* 2005). The thromboxane A2/TP_a receptor was demonstrated to activate β-catenin/TCF-dependent transcription apparently through the phospho-inhibition of GSK3β (Yan & Tai 2006; Fig. 1).

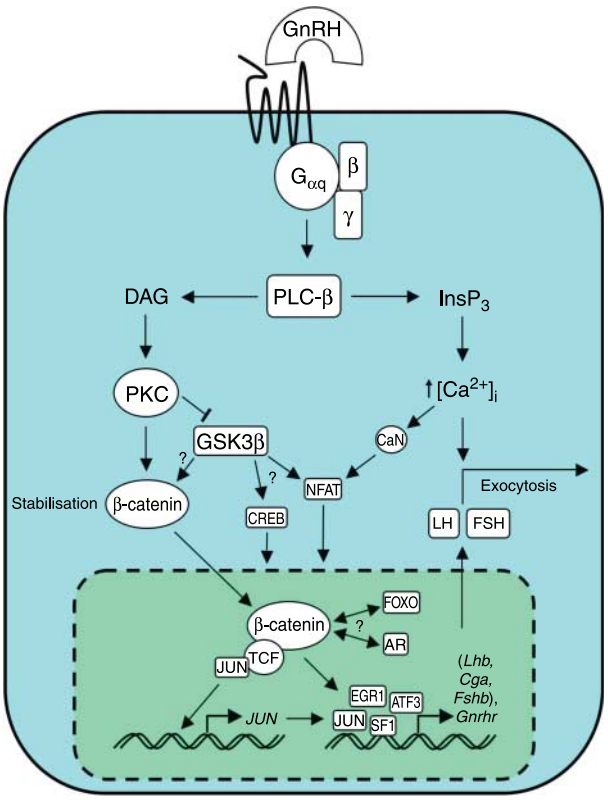

Figure 1 Targeting Wnt signalling mediators by GnRH in gonadotropes. GnRH activates PLC-β via coupling to $G_{q/11}$, resulting in the hydrolysis of membrane-bound phosphatidylinositol 4,5-bisphosphate into inositol 1,4,5-triphosphate ($InsP_3$) and diacylglycerol (DAG), which respectively mobilise intracellular Ca^{2+} and activate protein kinase C (PKC). GnRH targets Ser^9 phospho-inhibition of GSK3β through a PKC-mediated mechanism, which may in part be responsible for the stabilisation and accumulation of β-catenin levels. β-catenin translocates to the nucleus where it acts as a co-factor to TCF, to increase the transcription of TCF target genes, including *Fra1*, *Jun* and *Myc*. β-catenin also interacts with SF1, which together with other DNA-binding proteins (including EGR1, JUN and ATF3) increases transcription of *Lhb*, and possibly of *Cga*, *Fshb* and *Gnrhr*. In addition, β-catenin may act as a co-factor to regulate members of the FoxO family and the androgen receptor (AR). GSK3β may target the activities of other transcription factors in gonadotropes, including CREB and NFAT, which may therefore be subject to an additional level of regulation by GnRH, since GnRH inhibits GSK3β activity. See text for details.

The GnRH receptor targets GSK3β phospho-inhibition and β-catenin/TCF transcriptional activity

The above studies suggested that further cross-talk between signalling mediators of the Wnt/β-catenin pathway and those activated by GPCRs is likely. Indeed, several recent studies have demonstrated that GnRH, acting at the GnRH receptor, can target mediators of the Wnt/β-catenin signalling pathway in both a heterologous HEK293 model cell line and the LβT2 gonadotrope cell line (Gardner *et al.* 2007, Salisbury *et al.* 2007, 2008, 2009, Gardner & Pawson 2009).

The initial studies reported GnRH-mediated nuclear accumulation of β-catenin, activation of β-catenin/TCF-dependent transcription (using a TCF-dependent luciferase reporter plasmid) and the up-regulation of several β-catenin/TCF target genes including *Jun*, *Fra1* and *Myc* (Gardner *et al.* 2007). In addition, it was demonstrated that GnRH targets Ser^9 GSK3β phospho-inhibition (Gardner *et al.* 2007). As discussed above, the accumulation of β-catenin in response to GnRH may be independent of the Ser^9 phospho-inhibition of GSK3β since the Wnt pool of GSK3β is probably functionally

distinct from the pool of GSK3β that GnRH targets. The mechanism whereby GnRH targets β-catenin accumulation and Ser^9 GSK3β phospho-inhibition was shown to be mediated via $G_{q/11}$ coupling, and is most likely PKC dependent (Gardner *et al.* 2007). Furthermore, GnRH does not signal through PI3K–Akt/PKB to target GSK3β phospho-inhibition, as is the case in classical insulin signalling, since pharmacological inhibition of PI3K failed to block β-catenin/TCF transcriptional activity in response to GnRH (Gardner *et al.* 2007).

Apart from its role as a TCF co-factor in the canonical Wnt signalling pathway, β-catenin can function as a co-factor to a number of other transcription factors relevant to gonadotrope biology, including SF1 and JUN (Angel *et al.* 1988, Desclozeaux *et al.* 2002, Shah *et al.* 2002, Gummow *et al.* 2003, Mizusaki *et al.* 2003, Parakh *et al.* 2006). Thus, the ability of GnRH to stimulate the expression of β-catenin/TCF target genes suggests that SF1- and JUN-responsive genes may also be targets of GnRH-dependent β-catenin/TCF transcriptional activity. This is because functional cross-talk between SF1, JUN and β-catenin/TCF signalling has been demonstrated in several studies (Shah *et al.* 2002, Gummow *et al.* 2003, Mizusaki *et al.* 2003, Veeman *et al.* 2003, Le Floch *et al.* 2005). Furthermore, β-catenin acts as a co-factor of SF1 through a direct interaction (Gummow *et al.* 2003, Mizusaki *et al.* 2003, Parakh *et al.* 2006), while JUN can interact co-operatively with TCF in the β-catenin/TCF complex at JUN promoter sites (Nateri *et al.* 2005).

Targeting β-catenin/TCF transcriptional activity in gonadotropes

The ability of GnRH to stimulate the expression of β-catenin/TCF target genes, and the finding that both SF1- and JUN-responsive genes may also be targets of GnRH-dependent β-catenin/TCF transcriptional activity, has important implications for gonadotrope function, including the expression of *Lhb*, *Cga*, *Fshb* and *Gnrhr* genes. An important study highlighting the role of β-catenin as a member of a transcription factor complex that drives maximal activity of the *Lhb* subunit promoter in response to GnRH was published (Salisbury *et al.* 2007). This study by Nilson *et al.* demonstrates the co-localisation of β-catenin with SF1 and EGR1 on the promoter of the *Lhb* subunit gene in response to GnRH, and suggests that endogenous SF1 and β-catenin can physically associate in LβT2 cells (Salisbury *et al.* 2007). A role for GnRH targeting of β-catenin/TCF activity to regulate the expression of JUN-responsive genes including *Cga*, *Fshb* and *Gnrhr*, which additionally require SF1, is yet to be demonstrated. What is clear though is that β-catenin has an important role to play, and that it regulates gonadotrope responsiveness to GnRH.

Concluding remarks and future direction

The ability of GnRH to impinge on the activity of Wnt signalling mediators has several implications for further understanding key processes in gonadotrope biology. For example, CREB has a well-known role in regulating *Gnrhr* expression by targeting CRE sites within the *Gnrhr* promoter in response to GnRH stimulation (Cheng & Leung 2001, Maya-Nunez & Conn 2001). GSK3 has been proposed as one of a number of kinases that regulate CREB activity (Frame & Cohen 2001, Grimes & Jope 2001, Doble & Woodgett 2003). Thus, by promoting the Ser^9 phospho-inhibition of GSK3β, GnRH signal transduction may provide an additional level of complexity in the regulation of CREB activity and its ability to function optimally at the *Gnrhr* promoter. GSK3 can also target the activity of the Ca^{2+}-sensitive transcription factor NFAT by functioning as a nuclear export kinase, thereby terminating NFAT transcriptional activity (Crabtree & Olson 2002). GnRH has been shown to mediate the derepression of the *Fshb* gene in the αT3-1 gonadotrope cell line through the activation of the Ca^{2+}/CaN pathway leading to NFAT-driven expression of *Nur77* (Lim *et al.* 2007). Furthermore, a recent study has reported that GnRH-mediated Ca^{2+}/NFAT signalling does not act as the GnRH pulse frequency decoder in LβT2 cells (Armstrong *et al.* 2009). It will be interesting to determine if there is a role for GnRH-mediated Ser^9 phospho-inhibition of GSK3β in regulating NFAT nuclear residency and how this impacts on gonadotrope function with regard to these studies. As a multifunctional kinase with a diverse array of proposed targets, it is likely that many more roles for GSK3 in the gonadotropes will emerge.

Additional roles for β-catenin are also likely, including the regulation of androgen receptor (AR) activity. As an AR co-factor, β-catenin functions to both inhibit and stimulate AR target gene expression in a variety of cell types and tissues (Chesire & Isaacs 2003, Mulholland *et al.* 2005, Terry *et al.* 2006, Robinson *et al.* 2008). Expression of *Cga* (α-polypeptide glycoprotein hormones), *Lhb* and *Fshb* subunit gene is widely reported to involve a component of androgen regulation in gonadotropes; however, possible roles for β-catenin in modulating AR activity at this level (in response to GnRH) have not been reported yet (Curtin *et al.* 2001, Jorgensen & Nilson 2001*a*,*b*, Curtin *et al.* 2004, Spady *et al.* 2004, Thackray *et al.* 2006, Burger *et al.* 2007, Thackray & Mellon 2008). Furthermore, roles for FOXO transcription factors, which employ β-catenin as a co-factor, have been proposed (Essers *et al.* 2005, Malbon 2005, Hoogeboom *et al.* 2008, Jin *et al.* 2008, Hoogeboom & Burgering 2009, Stavrou *et al.* 2009). Although clearly speculative, elucidating potential roles for GSK3β and β-catenin would

enhance our understanding of the complexity of the regulation of gonadotropin subunit expression and gonadotrope function.

In conclusion, this review has highlighted both published and putative roles for mediators of the Wnt signalling pathways in targeting important biological processes in gonadotrope biology. We suggest that new avenues of research will continue to emerge in order to advance our understanding of the targeting of Wnt signalling mediators by GnRH to in turn regulate gonadotrope function. Indeed, such studies are already well underway.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

Angel P, Hattori K, Smeal T & Karin M 1988 The jun proto-oncogene is positively autoregulated by its product, Jun/AP-1. *Cell* **55** 875–885.

Armstrong SP, Caunt CJ, Fowkes RC, Tsaneva-Atanasova K & McArdle CA 2009 Pulsatile and sustained gonadotropin-releasing hormone (GnRH) receptor signaling: does the Ca^{2+}/NFAT signaling pathway decode GnRH pulse frequency? *Journal of Biological Chemistry* **284** 35746–35757.

Burger LL, Haisenleder DJ, Wotton GM, Aylor KW, Dalkin AC & Marshall JC 2007 The regulation of FSHβ transcription by gonadal steroids: testosterone and estradiol modulation of the activin intracellular signaling pathway. *American Journal of Physiology. Endocrinology and Metabolism* **293** E277–E285.

Caunt CJ, Finch AR, Sedgley KR & McArdle CA 2006 GnRH receptor signalling to ERK: kinetics and compartmentalization. *Trends in Endocrinology and Metabolism* **17** 308–313.

Cheng KW & Leung PC 2001 Human gonadotropin-releasing hormone receptor gene transcription: up-regulation by 3′,5′-cyclic adenosine monophosphate/protein kinase A pathway. *Molecular and Cellular Endocrinology* **181** 15–26.

Chesire DR & Isaacs WB 2003 β-Catenin signaling in prostate cancer: an early perspective. *Endocrine-Related Cancer* **10** 537–560.

Cook D, Fry MJ, Hughes K, Sumathipala R, Woodgett JR & Dale TC 1996 Wingless inactivates glycogen synthase kinase-3 via an intracellular signalling pathway which involves a protein kinase C. *EMBO Journal* **15** 4526–4536.

Crabtree GR & Olson EN 2002 NFAT signaling: choreographing the social lives of cells. *Cell* **109** S67–S79.

Curtin D, Jenkins S, Farmer N, Anderson AC, Haisenleder DJ, Rissman E, Wilson EM & Shupnik MA 2001 Androgen suppression of GnRH-stimulated rat LHβ gene transcription occurs through Sp1 sites in the distal GnRH-responsive promoter region. *Molecular Endocrinology* **15** 1906–1917.

Curtin D, Ferris HA, Hakli M, Gibson M, Janne OA, Palvimo JJ & Shupnik MA 2004 Small nuclear RING finger protein stimulates the rat luteinizing hormone-β promoter by interacting with Sp1 and steroidogenic factor-1 and protects from androgen suppression. *Molecular Endocrinology* **18** 1263–1276.

Desclozeaux M, Krylova IN, Horn F, Fletterick RJ & Ingraham HA 2002 Phosphorylation and intramolecular stabilization of the ligand binding domain in the nuclear receptor steroidogenic factor 1. *Molecular and Cellular Biology* **22** 7193–7203.

Ding VW, Chen RH & McCormick F 2000 Differential regulation of glycogen synthase kinase 3β by insulin and Wnt signaling. *Journal of Biological Chemistry* **275** 32475–32481.

Ding Q, Xia W, Liu JC, Yang JY, Lee DF, Xia J, Bartholomeusz G, Li Y, Pan Y, Li Z *et al.* 2005 Erk associates with and primes GSK-3β for its inactivation resulting in upregulation of β-catenin. *Molecules and Cells* **19** 159–170.

Dobkin-Bekman M, Naidich M, Pawson AJ, Millar RP, Seger R & Naor Z 2006 Activation of mitogen-activated protein kinase (MAPK) by GnRH is cell-context dependent. *Molecular and Cellular Endocrinology* **252** 184–190.

Doble BW & Woodgett JR 2003 GSK-3: tricks of the trade for a multi-tasking kinase. *Journal of Cell Science* **116** 1175–1186.

van Es JH, Barker N & Clevers H 2003 You Wnt some, you lose some: oncogenes in the Wnt signaling pathway. *Current Opinion in Genetics and Development* **13** 28–33.

Essers MA, de Vries-Smits LM, Barker N, Polderman PE, Burgering BM & Korswagen HC 2005 Functional interaction between β-catenin and FOXO in oxidative stress signaling. *Science* **308** 1181–1184.

Farias GG, Godoy JA, Hernandez F, Avila J, Fisher A & Inestrosa NC 2004 M1 muscarinic receptor activation protects neurons from β-amyloid toxicity. A role for Wnt signaling pathway. *Neurobiology of Disease* **17** 337–348.

Frame S & Cohen P 2001 GSK3 takes centre stage more than 20 years after its discovery. *Biochemical Journal* **359** 1–16.

Fujino H & Regan JW 2001 FP prostanoid receptor activation of a T-cell factor/β-catenin signaling pathway. *Journal of Biological Chemistry* **276** 12489–12492.

Fujino H, West KA & Regan JW 2002 Phosphorylation of glycogen synthase kinase-3 and stimulation of T-cell factor signaling following activation of EP2 and EP4 prostanoid receptors by prostaglandin E_2. *Journal of Biological Chemistry* **277** 2614–2619.

Gardner S & Pawson AJ 2009 Emerging targets of the GnRH receptor: novel interactions with Wnt signalling mediators. *Neuroendocrinology* **89** 241–251.

Gardner S, Maudsley S, Millar RP & Pawson AJ 2007 Nuclear stabilization of β-catenin and inactivation of glycogen synthase kinase-3β by gonadotropin-releasing hormone: targeting Wnt signaling in the pituitary gonadotrope. *Molecular Endocrinology* **21** 3028–3038.

Grimes CA & Jope RS 2001 The multifaceted roles of glycogen synthase kinase 3β in cellular signaling. *Progress in Neurobiology* **65** 391–426.

Gummow BM, Winnay JN & Hammer GD 2003 Convergence of Wnt signaling and steroidogenic factor-1 (SF-1) on transcription of the rat inhibin alpha gene. *Journal of Biological Chemistry* **278** 26572–26579.

Hoogeboom D & Burgering BM 2009 Should I stay or should I go: β-catenin decides under stress. *Biochimica et Biophysica Acta* **1796** 63–74.

Hoogeboom D, Essers MA, Polderman PE, Voets E, Smits LM & Burgering BM 2008 Interaction of FOXO with β-catenin inhibits β-catenin/T cell factor activity. *Journal of Biological Chemistry* **283** 9224–9230.

Huelsken J & Behrens J 2002 The Wnt signalling pathway. *Journal of Cell Science* **115** 3977–3978.

Jin T, George Fantus I & Sun J 2008 Wnt and beyond Wnt: multiple mechanisms control the transcriptional property of β-catenin. *Cellular Signalling* **20** 1697–1704.

Jorgensen JS & Nilson JH 2001*a* AR suppresses transcription of the alpha glycoprotein hormone subunit gene through protein–protein interactions with cJun and activation transcription factor 2. *Molecular Endocrinology* **15** 1496–1504.

Jorgensen JS & Nilson JH 2001*b* AR suppresses transcription of the LHβ subunit by interacting with steroidogenic factor-1. *Molecular Endocrinology* **15** 1505–1516.

Katoh M & Katoh M 2007 WNT signaling pathway and stem cell signaling network. *Clinical Cancer Research* **13** 4042–4045.

Kohn AD & Moon RT 2005 Wnt and calcium signaling: β-catenin-independent pathways. *Cell Calcium* **38** 439–446.

Kuhl M, Sheldahl LC, Park M, Miller JR & Moon RT 2000 The Wnt/Ca^{2+} pathway: a new vertebrate Wnt signaling pathway takes shape. *Trends in Genetics* **16** 279–283.

Le Floch N, Rivat C, De Wever O, Bruyneel E, Mareel M, Dale T & Gespach C 2005 The proinvasive activity of Wnt-2 is mediated through a noncanonical Wnt pathway coupled to GSK-3β and c-Jun/AP-1 signaling. *FASEB Journal* **19** 144–146.

Lim S, Luo M, Koh M, Yang M, bin Abdul Kadir MN, Tan JH, Ye Z, Wang W & Melamed P 2007 Distinct mechanisms involving diverse histone deacetylases repress expression of the two gonadotropin β-subunit genes in immature gonadotropes, and their actions are overcome by gonadotropin-releasing hormone. *Molecular and Cellular Biology* **27** 4105–4120.

Liu X, Liu T, Slusarski DC, Yang-Snyder J, Malbon CC, Moon RT & Wang H 1999 Activation of a frizzled-2/β-adrenergic receptor chimera promotes Wnt signaling and differentiation of mouse F9 teratocarcinoma cells via Galphao and Galphat. *PNAS* **96** 14383–14388.

Liu T, DeCostanzo AJ, Liu X, Wang H, Hallagan S, Moon RT & Malbon CC 2001 G protein signaling from activated rat frizzled-1 to the β-catenin–Lef–Tcf pathway. *Science* **292** 1718–1722.

Malbon CC 2005 β-Catenin, cancer, and G proteins: not just for frizzleds anymore. *Science's STKE* **2005** pe35.

Maya-Nunez G & Conn PM 2001 Cyclic adenosine 3′,5′-monophosphate (cAMP) and cAMP responsive element-binding protein are involved in the transcriptional regulation of gonadotropin-releasing hormone (GnRH) receptor by GnRH and mitogen-activated protein kinase signal transduction pathway in GGH(3) cells. *Biology of Reproduction* **65** 561–567.

McManus EJ, Sakamoto K, Armit LJ, Ronaldson L, Shpiro N, Marquez R & Alessi DR 2005 Role that phosphorylation of GSK3 plays in insulin and Wnt signalling defined by knockin analysis. *EMBO Journal* **24** 1571–1583.

Millar RP, Lu ZL, Pawson AJ, Flanagan CA, Morgan K & Maudsley SR 2004 Gonadotropin-releasing hormone receptors. *Endocrine Reviews* **25** 235–275.

Millar RP, Pawson AJ, Morgan K, Rissman EF & Lu ZL 2008 Diversity of actions of GnRHs mediated by ligand-induced selective signaling. *Frontiers in Neuroendocrinology* **29** 17–35.

Mizusaki H, Kawabe K, Mukai T, Ariyoshi E, Kasahara M, Yoshioka H, Swain A & Morohashi K 2003 Dax-1 (dosage-sensitive sex reversal-adrenal hypoplasia congenita critical region on the X chromosome, gene 1) gene transcription is regulated by wnt4 in the female developing gonad. *Molecular Endocrinology* **17** 507–519.

Moon RT, Kohn AD, De Ferrari GV & Kaykas A 2004 WNT and β-catenin signalling: diseases and therapies. *Nature Reviews. Genetics* **5** 691–701.

Mulholland DJ, Dedhar S, Coetzee GA & Nelson CC 2005 Interaction of nuclear receptors with the Wnt/β-catenin/Tcf signaling axis: Wnt you like to know? *Endocrine Reviews* **26** 898–915.

Naor Z 1997 GnRH receptor signaling: cross-talk of Ca^{2+} and protein kinase C. *European Journal of Endocrinology* **136** 123–127.

Naor Z 2009 Signaling by G-protein-coupled receptor (GPCR): studies on the GnRH receptor. *Frontiers in Neuroendocrinology* **30** 10–29.

Naor Z, Benard O & Seger R 2000 Activation of MAPK cascades by G-protein-coupled receptors: the case of gonadotropin-releasing hormone receptor. *Trends in Endocrinology and Metabolism* **11** 91–99.

Nateri AS, Spencer-Dene B & Behrens A 2005 Interaction of phosphorylated c-Jun with TCF4 regulates intestinal cancer development. *Nature* **437** 281–285.

Nelson WJ & Nusse R 2004 Convergence of Wnt, β-catenin, and cadherin pathways. *Science* **303** 1483–1487.

Ng SS, Mahmoudi T, Danenberg E, Bejaoui I, de Lau W, Korswagen HC, Schutte M & Clevers H 2009 Phosphatidylinositol 3-kinase (PI3K) signaling does not activate the Wnt cascade. *Journal of Biological Chemistry* **284** 35308–35313.

Parakh TN, Hernandez JA, Grammer JC, Weck J, Hunzicker-Dunn M, Zeleznik AJ & Nilson JH 2006 Follicle-stimulating hormone/cAMP regulation of aromatase gene expression requires β-catenin. *PNAS* **103** 12435–12440.

Pawson AJ & McNeilly AS 2005 The pituitary effects of GnRH. *Animal Reproduction Science* **88** 75–94.

Polakis P 2000 Wnt signaling and cancer. *Genes and Development* **14** 1837–1851.

Robinson DR, Zylstra CR & Williams BO 2008 Wnt signaling and prostate cancer. *Current Drug Targets* **9** 571–580.

Salisbury TB, Binder AK, Grammer JC & Nilson JH 2007 Maximal activity of the luteinizing hormone β-subunit gene requires β-catenin. *Molecular Endocrinology* **21** 963–971.

Salisbury TB, Binder AK & Nilson JH 2008 Welcoming β-catenin to the gonadotropin-releasing hormone transcriptional network in gonadotropes. *Molecular Endocrinology* **22** 1295–1303.

Salisbury TB, Binder AK, Grammer JC & Nilson JH 2009 GnRH-regulated expression of Jun and JUN target genes in gonadotropes requires a functional interaction between TCF/LEF family members and β-catenin. *Molecular Endocrinology* **23** 402–411.

Schulte G & Bryja V 2007 The Frizzled family of unconventional G-protein-coupled receptors. *Trends in Pharmacological Sciences* **28** 518–525.

Shah S, Pishvaian MJ, Easwaran V, Brown PH & Byers SW 2002 The role of cadherin, β-catenin, and AP-1 in retinoid-regulated carcinoma cell differentiation and proliferation. *Journal of Biological Chemistry* **277** 25313–25322.

Spady TJ, Shayya R, Thackray VG, Ehrensberger L, Bailey JS & Mellon PL 2004 Androgen regulates follicle-stimulating hormone β gene expression in an activin-dependent manner in immortalized gonadotropes. *Molecular Endocrinology* **18** 925–940.

Stambolic V & Woodgett JR 1994 Mitogen inactivation of glycogen synthase kinase-3β in intact cells via serine 9 phosphorylation. *Biochemical Journal* **303** 701–704.

Stavrou E, Millar RP & Pawson AJ 2009 GnRH regulation of FoxO transcription factors. Society for Endocrinology BES 2009, Harrogate, UK. *Endocrine Abstracts* **19** P110.

Terry S, Yang X, Chen MW, Vacherot F & Buttyan R 2006 Multifaceted interaction between the androgen and Wnt signaling pathways and the implication for prostate cancer. *Journal of Cellular Biochemistry* **99** 402–410.

Thackray VG & Mellon PL 2008 Synergistic induction of follicle-stimulating hormone β-subunit gene expression by gonadal steroid hormone receptors and Smad proteins. *Endocrinology* **149** 1091–1102.

Thackray VG, McGillivray SM & Mellon PL 2006 Androgens, progestins, and glucocorticoids induce follicle-stimulating hormone β-subunit gene expression at the level of the gonadotrope. *Molecular Endocrinology* **20** 2062–2079.

Thomas GM, Frame S, Goedert M, Nathke I, Polakis P & Cohen P 1999 A GSK3-binding peptide from FRAT1 selectively inhibits the GSK3-catalysed phosphorylation of axin and β-catenin. *FEBS Letters* **458** 247–251.

Veeman MT, Axelrod JD & Moon RT 2003 A second canon. Functions and mechanisms of β-catenin-independent Wnt signaling. *Developmental Cell* **5** 367–377.

Yan W & Tai HH 2006 Glycogen synthase kinase-3 phosphorylation, T-cell factor signaling activation, and cell morphology change following stimulation of thromboxane receptor α. *Journal of Pharmacological and Experimental Therapeutics* **317** 267–274.

Yang M, Zhong WW, Srivastava N, Slavin A, Yang J, Hoey T & An S 2005 G protein-coupled lysophosphatidic acid receptors stimulate proliferation of colon cancer cells through the β-catenin pathway. *PNAS* **102** 6027–6032.

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